

# Toxicology Studies of Irradiation-Sterilized Chicken

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## ABSTRACT

Results of nutritional, genetic, and toxicological studies of shelf-stable chicken sterilized by ionizing radiation are presented. No evidence of genetic toxicity or teratogenic effects in mice, hamsters, rats, and rabbits was observed. There was an unexplained reduction in the hatchability of eggs of *Drosophila melanogaster* reared on gamma-irradiated meat. No treatment-related abnormalities or changes were observed in dogs, rats, or mice during multigeneration studies. These nutritional, genetic, and toxicological studies did not provide definitive evidence of toxicological effects in mammals due to ingestion of chicken meat sterilized by ionizing radiation.

This report presents results of comprehensive nutritional, genetic, and toxicological studies of chicken sterilized by ionizing radiation which were initiated by the U.S. Army in 1976 and completed in 1984. From 1940 to 1953 exploratory research in food irradiation was sponsored by the Department of the Army, the Atomic Energy Commission, and by private industry (12,13). Industrial scientists in 1955 reported the results of a multigeneration feeding study in which three generations of albino rats (2685 animals) were fed electron irradiation-sterilized raw ground beef or non-irradiated beef (30). There was no evidence of toxicity or decrease in nutritional value of the raw meat due to the irradiation treatment. The U.S. Army tested 54 irradiated food items representing virtually every food class in a series of short-term acute toxicity studies (9,10,13,23,32,35,39). No toxic effects were identified and a second phase was initiated consisting of short-term studies with human volunteers. A total of seven 15-d tests were conducted in which irradiated foods constituted 32 to 100% of the caloric intake of the test subjects (29). At the start, dur-

ing, at completion, and 1 year after completing clinical laboratory and physical examinations were carried out. No adverse effects were noted from consumption of the irradiated foods (29). In 1956 systematic, long-term animal feeding studies were initiated to determine the toxicity and nutritional quality of 22 representative irradiated foods (12). These foods were fed to rats, dogs, mice, and monkeys for 2 years to determine possible chronic toxicological effects, carcinogenicity, and nutritional adequacy. The effects of ingestion of these foods on growth, reproduction, longevity, lactation, tumor incidence, histopathology, and evidence of metabolic changes were assessed. These tests continued through 1964 and conformed to the best current (pre-GLP) standards. Problems unrelated to safety were encountered. In 1965 it was concluded that these problems were related to animal nutrition and not to the irradiation processing of foods, and further, that food irradiated to an absorbed dose of 56 kGy was safe and nutritionally adequate (12,31).

The U.S. Army petitioned the FDA for clearance of raw bacon, packed in vacuo and irradiation sterilized (45 to 56 kGy at 5°C); clearance for this product was granted in February 1963. Additional clearances were granted for irradiation of wheat and wheat products for insect disinfestation in August 1963, and for irradiation of white potatoes for sprout inhibition in June 1964.

In 1968 the FDA rescinded the approval of the irradiated canned bacon regulation, stating that a careful analysis of all submitted data indicated significant adverse effects in animals fed irradiated food, and that major deficiencies existed in the design rather than the conclusions of some experiments (4,31,36). There was a 20.7% decrease in surviving weaned young in rats fed a diet containing bacon irradiated with 27.9 kGy and those rats fed a diet containing bacon irradiated with a 55.8 kGy dose had a 28.7% decrease in surviving weaned young when compared with animals on the unirradiated diet (4,31,38).

The FDA and the National Research Council of the National Academy of Sciences cooperated with army scientists to develop new protocols for greatly expanded animal feeding studies of irradiated beef, ham, pork, and chicken (1,6,31). The study of the nutritional value and

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wholesomeness of chicken products sterilized with ionizing radiation was contracted to Raltech Scientific Services of St. Louis, Missouri in 1976; however, additional work was performed as part of the study at several other institutions. Those studies are reviewed in the following paragraphs since they form an essential part of the wholesomeness studies.

The detrimental effects of ionizing radiation, even at cryogenic temperatures, on the content of the water-soluble vitamins niacin, thiamin, riboflavin, and pyridoxin in meats is well documented (2,18,19,34). Accordingly, studies were conducted at the Letterman Army Institute of Research to determine if freezing, thermal processing, or ionizing radiation produced factors which were antagonistic to vitamins B<sub>1</sub> and B<sub>6</sub> in the diets of rats. To identify possibly antithiamine properties of the test diets, male and female weanling rats (156 each) were made thiamine-deficient on a semi-purified diet lacking thiamine. The animals were then repleted with the various test diets containing chicken and either marginal (3 mg/kg) or high (20 mg/kg) levels of thiamine. Recovery rates were monitored by weight gain and measurements of erythrocyte transketolase (26,27). In a similar study, to determine if freezing, thermal processing, or ionizing radiation produced factors antagonistic to pyridoxine (B<sub>6</sub>) male and female rats (156 each) were made vitamin B<sub>6</sub>-deficient by feeding a semi-purified diet devoid of the vitamin. The animals were repleted with the various test diets containing either marginal (2.5 mg pyridoxine•HCl/kg) or high (12 mg/kg) levels of vitamin B<sub>6</sub>. Recovery rates were monitored by growth (weight gain) and measurements of pyridoxine-dependent blood enzymes (plasma and red cell aspartate aminotransferase and alanine aminotransferase). Experimental details are available from the National Technical Information Service (28). No evidence of antithiamine substances was found in either gamma- or electron-radiation sterilized chicken (26,27). No evidence was found for anti-vitamin B<sub>6</sub> activity in electron-irradiated chicken and minimal activity was found in gamma irradiated chicken (28).

Responsibility for supervision of the Raltech Scientific Services contract was transferred from the U.S. Army to the Department of Agriculture in October 1980. The reports were reviewed and accepted in their final form in 1984. This manuscript reports the major findings of the studies conducted at Raltech Scientific Services.

## MATERIALS AND METHODS

### *Irradiation technology*

The cobalt-60 and 10 MeV electron accelerator food irradiation facilities and their operation at the U.S. Army's Natick Research Development Laboratories was described by MacDonald et al. (25). The dosimetry which was used in support of the toxicological studies was described by Jarrett and Halliday (17). The actual operational conditions and citation of dosimetry records during the irradiation of the chicken products was described by Wierbicki (40,41).

### *Packaging technology*

Descriptions of cans and flexible packages, their use, limits, and evaluations for packaging of the chicken products used for the toxicological studies were determined by Killoran and co-workers (20-22). The frozen gamma- and thermally-sterilized chicken products were canned in vacuo in 404 × 309 mm epoxy-phenolic-enamel lined cans. The flexible packages were 165 mm × 208 mm and fabricated with 0.025 mm polyiminocaproyl (nylon 6) as the outside layer, 0.0090 mm aluminum foil as the middle layer, and 0.051 mm polyethylene terephthalate polyethylene as the food contacting layer. The pouches were vacuum-sealed (41).

### *Processing of chicken meat*

A total of 135,405 kg of 1.36 to 1.59 kg carcass weight broilers (230,000 chickens) were processed for the studies. The carcasses were hand deboned and the skins with subcutaneous fat were separated from the meat. The blended product contained 18% skin and 82% lean meat with a total fat content of 12-13%. To each 100 kg of meat and skin were added 0.75 kg of NaCl and 0.30 kg of sodium tripolyphosphate. The meat, skin, and additives were mixed in vacuo, stuffed with cellulose casings, and heated to an internal temperature of 73-80°C to inactivate enzymes. This process required 9-11 h and yielded 87% of the formula weight. Complete details of the poultry processing were described by Wierbicki (40,41).

Meat which was to be used as a frozen control (FC) was canned in vacuo and then frozen. Meat which was to be used as a thermally processed (TP) control was canned in vacuo and thermally processed at 115.6°C to a sterility level of F<sub>0</sub>=6. Diet GAM contained the enzyme-inactivated chicken meat which was canned in vacuo and sterilized by exposure to gamma radiation at -25 ± 15°C from a <sup>60</sup>Co source. The minimum absorbed dose was 46 kGy; the maximum absorbed dose was 68 kGy at an average dose rate of 578 Gy/min. The dose rate was 670 Gy/min in May 1976 when the first product was sterilized and had decreased to 521 Gy/min when the last batch of product was sterilized. Diet ELE contained the enzyme-inactivated chicken which had been vacuum-packed in 26-mm thick slices in laminated foil packages and sterilized by exposure to 10 MeV electrons at -25°C ± 15°C. The minimum absorbed dose was 45 kGy and the maximum 68 kGy with the average 58 kGy.

### *Protocol*

The nutritional, genetic, and toxicological studies fall into four general classes: (a) nutrition studies; (b) teratology studies; (c) chronic toxicity, oncogenicity, and multigeneration reproductive studies; and (d) genetic toxicity studies. The complete protocols for the toxicology studies are available from the National Technical Information Service (1,6.). Additional experimental detail may be found in each technical report.

### *Diets*

Five diets were evaluated, designated CLD, FC, TP, GAM, ELE. CLD was the negative or husbandry control diet and served as the carrier for the chicken meat in the other four diets. Diet CLD was the commercially available ration from the Ralston Purina Company appropriate for the species under study: Formulab Chow for rodents (CD-1 mice, Sprague-Dawley rats, Syrian Golden hamsters), Rabbit Chow for New Zealand White rabbits, and Lab Canine Diet for beagle dogs. Diets FC, TP, GAM, and ELE each contained, on a dry weight basis,

65% of the CLD diet and 35% chicken meat processed as described above.

The test chicken meats for the nutritional studies (FC, TP, GAM, and ELE) were lyophilized and processed through a food cutter (Model 8181D, Hobart Manufacturing Co., Troy, OH) to achieve uniformity in particle size and incorporated into the diet formulation to average 12.0% protein on a dry weight basis. The diet formulations conformed to the 1975 AOAC (5) specifications with the exceptions that the vitamin mix was stabilized by the addition of 1.0% butylated hydroxytoluene (BHT); a modification of the mineral mix of Wilcke et al. (42) was used, and the fiber content was adjusted to 1.6% rather than to 1.0% because of the fiber content of the CLD.

#### Nutritional studies

The nutritional studies examined the protein efficiency ratios (PER) for rats and mice and evaluated the possible antivitamin effects of the irradiation sterilization of meats. ANRC Casein was added as the reference standard diet for evaluation of PER using 10 male and 10 female weanling (58-62 g) Sprague-Dawley rats housed individually for each of the six dietary protein sources (35). The PER of each test diet was calculated as the grams of weight gain per gram of protein intake over the 28-d test period. A separate PER value was obtained for each animal. These values were averaged for each sex by diet group.

#### Genetic toxicology studies

Four genetic toxicology studies were conducted by Raltech Scientific Services. In the first, the plate incorporation protocol for the *Salmonella*/mammalian microsome mutagenicity assay (3) using *Salmonella typhimurium* was modified since the test meat contained histidine (Ames et al., 1979. Supplement to the methods paper. Received by direct mail with test cultures from laboratory of B. Ames). The tests employed *S. typhimurium* TA1535, TA1537, TA1538, TA100, and TA98. Strain TA1535 detects mutagens which cause base-pair substitutions. Strains TA1537, TA1538, TA100, and TA98 detect various kinds of frameshift mutagens. Samples of chicken were homogenized in a Waring blender, and 10-g samples were blended in a Sorval Omini-mixer for 2 min with 19 ml of sterile deionized water plus 1 ml of dimethylsulfoxide (DMSO) solution (1/3 DMSO, 2/3 deionized water) containing the known mutagen (positive control) or with 20 ml of sterile deionized water without mutagen or DMSO solvent (test samples). The homogenate was centrifuged at 4°C for 90 min and the supernatant fluid filtered through sterile glass wool. The positive control mutagens were prepared to give approximately the same concentrations as those expected in the spiked chicken meat extracts with the same ratio of DMSO to water. The positive controls were 4-nitroquinoline-N-oxide ((6.25 µg/ml for control and 12.5 µg/ml of reaction mixture in chicken extract), 9-aminoacridine (100 µg/ml for control and 500 µg of reaction mixture for chicken extract), 2-aminofluorene (100 µg/ml reaction mixture), N-methyl-N'-nitro-N-nitrosoguanidine (10 µg/ml for control and 20 µg/ml of reaction mixture for chicken extract), and benzo(a)pyrene (40 µg/ml of reaction mixture). Appropriate concentrations of mutagens were determined by preliminary experiments (24). The reaction mixture consisted of 1 ml of an overnight culture of the tester strain in Oxoid Nutrient Broth No. 2 ( $1-2 \times 10^9$  viable cells/ml); 1 ml of the chicken extract filtrate or mutagen in DMSO solution; and when required, 1 ml of S-9 mixture. The reaction mixture was mixed with a Vortex Genie-mixer and incubated at room temperature (22°C) for 2 h. At the end of

the incubation period 25 ml of sterile physiological saline solution were added to each centrifuge tube and thoroughly mixed. The tubes were centrifuged for 30 min; the clear supernatant liquid was decanted; and the packed cells were brought to the original volume (1 ml) with sterile physiological saline solution. A 0.1-ml portion of the suspension was incorporated into the top agar and plated. Triplicate plates were prepared for each test solution. The plates were incubated for 48 h at 37°C and the entire test was repeated on a separate day. This extraciton procedure was developed from a series of preliminary experiments (24). The criterion was to use the procedure that yielded the optimum concentration of mutagen recovery from the chicken that gave a positive result and minimized the toxicity to the tester strains.

To test for sex-linked recessive lethal mutations, Canton-S type male *Drosophila melanogaster* were exposed to the test diets as they developed from eggs through the three larval instars to pupae and through adulthood (37). To expose the larval stages of the flies, the blended chicken meats were incorporated into Raltech Scientific Service's standard *Drosophila* medium at a concentration of 37.5% (wet weight). The final composition of this medium, excluding the pureed chicken, was agar, 9 g/kg; ground yellow-corn meal, 80 g/kg; brewer's yeast, 59 g/kg; unsulphured molasses, 137 g/kg; water, 713 g/kg; and propionic acid, 2 g/kg. The cool culture medium in 180-ml polypropylene specimen bottles was sprayed with an active culture of baker's yeast which was allowed to grow for 72 h before introduction of the flies. Approximately 25 female and 15 male Canton-S *Drosophila* adults were placed in each culture bottle, allowed to mate, and to lay their eggs on the media. The parent flies were removed at 7 d leaving the resulting larvae to ingest the media and to proceed through the three instars of development to pupae. The adult flies emerging from the pupae were exposed again to the test materials. The adults (collected 1 d after emergence) were fed a solution of 50% chicken meat and 1% fructose in water for 72 h. The positive control for both adults and larvae was 100 ppm tris(2,3-dibromopropyl)phosphate (TRIS). The Canton-S males were mated individually after treatment to three first multiple number 6 females (FM6) which contain a homozygous X-chromosome carrying phenotypic markers for yellow body, bar-shaped eye, and white colored eye, and several superimposed structural inversions which prevented "crossing over" with a homologous non-inverted X. The males were successively mated to three new FM6 virgin females until four groups of progeny were obtained from each male. When the four broods of offspring hatched, the daughters of the treated Canton-S males were mated individually to their FM6 male siblings for the lethal test. Fifteen adult males were treated with each chicken sample. Twenty-five offspring from each of the four broods produced by the treated males were mated, yielding about 1,500 tests for each sample. This was repeated six times, yielding approximately 9000 tests for sex-linked recessive lethal mutations per diet.

#### Teratology studies

Teratology studies were conducted with mice, hamsters, rats, and rabbits (11,14,15,16). Pregnant females were exposed to the test meats, at 35% and 70% (dry weight) of the total diet, during the respective period of maximum organogenesis. Twenty confirmed (by vaginal plug) pregnant CD-1 mice per diet group (total 240 mice) were provided with the test and control diets from day 1 through day 18 of gestation. Thirty Golden Syrian hamsters per diet group (total 360 animals) were

PROTOCOL  
MOUSE REPRODUCTION, CHRONIC TOXICITY  
(ALBINO, CD-1)

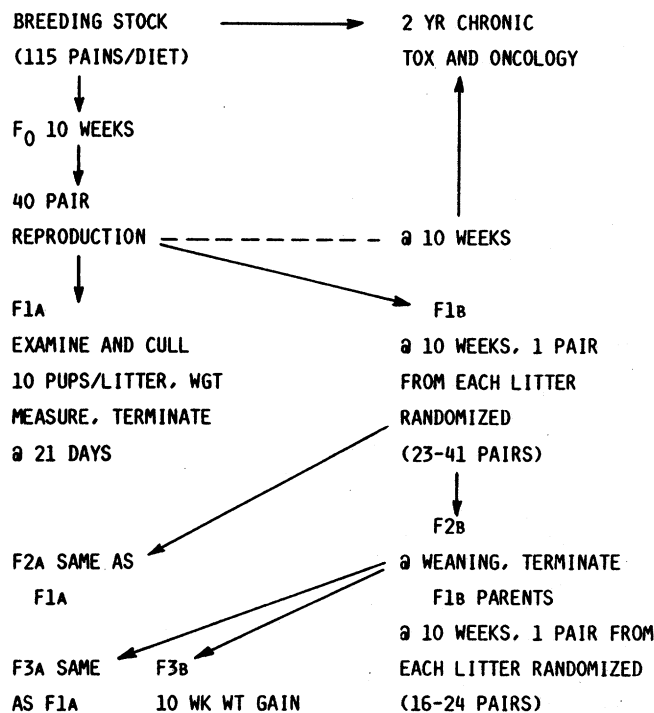


Figure 1. Protocol for the mouse chronic toxicity, oncogenicity, and multigeneration reproductive study.

provided with the test and control diets from days 6-10 of gestation. Thirty-three Sprague-Dawley pregnant rats per diet group (total 396) were provided with the test and control diets from days 1-20 of gestation. Twenty pregnant New Zealand white strain rabbits were provided with the test and control diets from days 6-18 of gestation. The positive control substances were all-trans retinoic acid for mice, hamsters, and rats, and thalidomide for rabbits. At laparotomy the number of live and dead fetuses, early and late resorptions, number of implantation sites, gross external abnormalities, and the internal development of each fetus were examined according to the procedures of Wilson and Warkany (43).

#### Chronic feeding studies

Chronic feeding studies were conducted at Raltech in mice and dogs using the five test diets provided ad lib. A thorough analysis of the nutrient content of the meats and diets was conducted throughout the period of study. No significant differences were found among the four test meats or the four meat-containing diets. Beagle dogs were exposed to the test or control diet beginning in utero until death or sacrifice at 36 months postweaning for females and 40 months postweaning for males (7). The study was designed to measure both chronic toxicity and breeding performance. Urine and blood specimens were collected from all F<sub>0</sub> dogs at weaning and at 3, 6, 9, 12, 18, 24, 30, and 36 months on test. Males were sampled again at 40 months on test. Analytical methods are described in the technical report (7). As the F<sub>0</sub> females attained sexual maturity, they were bred on successive estrus periods to produce the maximum number of litters before the end of the study. Offspring were selected from the F<sub>1</sub> generation litters at weaning for continued feeding to 6 months of age (Fig. 1). CD-1 mice

PROTOCOL  
BEAGLE REPRODUCTION, CHRONIC TOXICITY

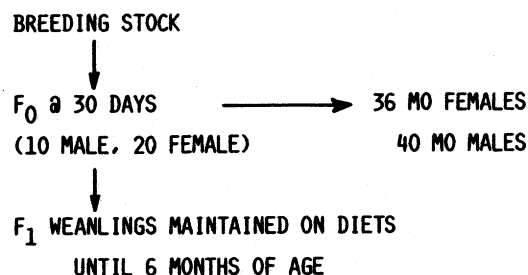


Figure 2. Protocol for the dog chronic toxicity, oncogenicity, and multigeneration reproductive study.

were exposed to the test and control diets beginning in utero and continuing until death or scheduled termination (8). The study design is presented in Fig. 2. The F<sub>0</sub> generation was continued on the test and control diets for 24 months postweaning. Cohorts of the F<sub>0</sub> mice were bred to begin the multigeneration reproduction study, then returned to the chronic feeding study after weaning of the F<sub>1b</sub> litter. This created identifiable subpopulations in the F<sub>0</sub> generation.

The PER values of all diets containing chicken were higher than the PER values of the casein standard for both males and female rats (Table 1). The PER values were not affected adversely by any of the methods by which the chicken was processed. The average cumulative 28-d feed consumption per rat among the males was lowest for animals on the casein control (294 g) and highest for animals fed the TP diet (326 g). Consumption was higher among the rats fed chicken diets than among those fed the casein control or CLD diets. Statistical analysis of the PER data, excluding the CLD diet, indicated that the PER of the males fed the TP diet was significantly lower ( $p < 0.01$ ) than for other groups receiving the chicken diets. Female rats fed the ELE diet had an average PER significantly higher than those receiving the FC diet ( $p < 0.04$ ) and TP diet ( $p < 0.02$ ). In both males and females receiving the FC diet, the PER was significantly higher than for those animals receiving the casein control diet.

It was concluded that the manner in which the chicken meat was processed, with or without radiation, had no effect on the response of the Salmonella-microsomal mutagenicity test system to known mutagens (24). In all cases, the untreated negative controls and solvent controls yielded revertant counts within or slightly lower than the normal spontaneous revertant ranges (Table 2). All of the positive mutagen controls yielded revertant count increases of two-fold or more over the untreated controls, indicating that the test cultures were performing satisfactorily. The standard mutagens from all of the chicken samples were recovered, although the results varied depending on the mutagen. 4-Nitroquinoline-N-oxide was recovered at the same rate as the mutagen control. 9-Aminoacridine was recovered at approximately 10 times

TABLE 1. Growth and protein efficiency ratios (PER) for rats fed the test diets.

Diet	Total wt gained (g)	Total feed consumed (g)	Total protein consumed (g)	Calculated 28-day PER
<b>Males</b>				
Casein	100	294	37.2	2.69 <sup>a</sup>
FC	123	324	37.5	3.28 <sup>b</sup>
TP	115	326	40.1	2.87 <sup>c,d</sup>
GAM	116	313	36.1	3.21 <sup>e(NS)</sup>
ELE	119	318	36.9	3.22
CLD	97	307	38.0	2.55
<b>Females</b>				
Casein	90	288	36.3	2.48 <sup>a</sup>
FC	95	302	35.2	2.70 <sup>b(NS)</sup>
TP	104	319	38.8	2.68
GAM	97	303	35.0	2.77
ELE	97	296	33.9	2.86
CLD	93	324	40.1	2.32

Comparisons: a (Casein to FC), b (FC to TP), c (TP to GAM), d (TP to ELE), e (GAM to ELE). Significantly ( $p < 0.01$ ) different from PER for diet compared (Student t-test). NS: p value greater than 0.50.

the positive control. The recoveries of benzo(a)pyrene, and N-methyl-N'-nitrosoquandine were 18% and that of 9-aminofluorene was 10%. All of the recoveries were greater than the negative control. However, the recovery of benzo(a)pyrene from the chicken yielded a revertant count only 1.4 times that of the negative control, and this recovery is regarded as partial. A positive result was not obtained from any of the chicken diets in the absence of an added mutagen. All of these results were at, or very close to, the negative controls.

None of the four processed chicken meats produced evidence of sex-linked recessive lethal mutations in *Drosophila melanogaster*, while the positive control, tris-

(2,3-dibromopropyl) phosphate, gave a significant mutagenic response (37). The numbers of observed recessive lethals per number of non-lethals observed during the study for each of the test diets were: negative control 14/8639, FC 10/8165, TP 15/8631, GAM 11/8609, ELE 12/8292, and TRIS 206/7146. In this same study there was, however, a significant reduction in the egg hatchability of cultures of *D. melanogaster* reared on gamma-irradiated chicken meat (GAM).

Additional testing was carried out to confirm these results and to attempt to determine the cause of the decreased number of offspring from cultures containing GAM. A different production lot of GAM was used to test the repeatability of the results. At a level of 37.5%, wet weight, the average numbers of offspring and the associated standard deviations ( $n = 5$ ) were: GAM-same lot, 25 (5); GAM-different lot, 45 (14); FC, 234 (66); and negative control 680 (121).

A comparison of dose response to diets FC and GAM produced the following results: FC-5%, 615 (179); FC-25%, 431 (107); FC-50%, 367 (143), GAM-5%, 498 (120); GAM-25%, 59 (39); GAM-37.5%, 25 (5); GAM-50%, 9 (5); negative control, 680 (121).

A different base medium was tried to see if this variable affected the results. The average numbers of offspring and the associated standard deviations ( $n = 5$ ) for the Raltech medium were negative control, 680 (121); FC, 234 (66); TP, 139 (83); ELE, 42 (19); GAM, 38 (35). Similar results were obtained using the new media: negative control, 478 (66); FC, 253 (247); TP, 46 (75); ELE, 128 (49); and GAM, 1 (1).

Vitamin supplementation of the medium did not result in any improvement in number of offspring. The results were negative control, 680 (121); negative control + vitamins, 658 (179); FC, 234 (66); FC + vitamins, 119 (42); GAM, 38 (35); GAM + vitamins, 2 (2).

TABLE 2. Ames mutagenicity pre-incubation test results with frozen, thermally processed, and gamma- or electron-irradiated chicken.

Test material, mutagen <sup>a</sup> test strain	Average revertant counts per plate $n = 6$ , standard deviation in parenthesis				
	MNNG TA 1535	NQNO TA 98	BP TA 100	9-AC TA 1537	9-AF TA 1538
Mutagen control	1631 (36)	415 (44)	253 (21)	45 (5)	1205 (25)
1/3 DMSO, 2/3 H <sub>2</sub> O	17 (2)	NA	101 (6)	5 (2)	10 (1)
Saline solution control	16 (1)	20 (4)	100 (7)	7 (2)	13 (2)
<b>Chicken without mutagen</b>					
Frozen (FC)	17 (4)	20 (3)	98 (13)	6 (4)	13 (2)
Thermal (TP)	17 (3)	20 (3)	104 (7)	6 (2)	9 (2)
Gamma (GAM)	17 (1)	23 (2)	107 (12)	6 (3)	11 (3)
Electron (ELE)	18 (3)	22 (4)	100 (11)	6 (3)	12 (4)
<b>Chicken with mutagen</b>					
Frozen	291 (181)	472 (56)	134 (11)	472 (182)	128 (14)
Thermal	251 (39)	469 (52)	140 (13)	475 (190)	144 (4)
Gamma	192 (19)	466 (61)	138 (7)	422 (145)	113 (11)
Electron	434 (269)	471 (54)	141 (15)	452 (182)	133 (15)

<sup>a</sup>MNNG = N-methyl-N'-nitro-N-nitrosoguanidine, NQNO = 4-nitroquinoline-N-oxide, BP = benzo(a)pyrene (with S-9 mix added), 9-AC = 9-aminoacridine, 9-AF = 9-aminofluorene (with S-9 mix added), NR: Not run.

It was concluded that although irradiated chicken was not mutagenic in this test system, there was a consistent reduction in number of offspring from *Drosophila* fed diets containing chicken. This effect was most pronounced with diets containing gamma irradiated chicken, and was dose-related. The cause of the reduction in number of offspring and the biological significance of these results with *Drosophila*, as they relate to man, is unknown.

In vivo studies in mice (Table 3), rats (Table 4), hamsters (Table 5), and rabbits (Table 6) led to the conclusion that none of the four processed chicken meat diets (FC, TP, GAM, or ELE) induced a teratogenic response when fed to the pregnant animals. The administration of positive controls (all-trans retinoic acid for mice, hamsters, and rats, and thalidomide for rabbits) induced, as expected, significant incidences of resorbed embryos and congenital malformations in both soft and skeletal body tissues; ingestion of any of the processed chicken meat diets (FC, TP, GAM, or ELE) did not induce significant incidences of resorbed embryos or congenital

malformations. Additional details of the experimental results are available in the technical reports (11,14,15,16).

All five diets supported growth of beagles to maturity, although group mean body weights and food consumption in F<sub>0</sub> and F<sub>1</sub> dogs fed diet CLD were significantly lower throughout life than those in groups fed the meat-containing diets (Table 7). No overt signs of diet-related toxicity were observed in any of the experimental groups. Male F<sub>0</sub> dogs fed the gamma-irradiated chicken (GAM) diet had significantly lower body weights through adulthood than males fed the frozen control chicken (FC). Mean body weights in group GAM males, however, did not differ significantly from groups TP and ELE. Many males fed diet FC became obese so that difference in body weight between groups FC and GAM was not considered evidence of toxicity. No overt signs of diet-related toxicity were observed in F<sub>0</sub> or F<sub>1</sub> dogs in any experimental group. Hematological, clinical biochemical, and histopathological findings in F<sub>0</sub> and F<sub>1</sub> dogs were unremarkable with respect to any treatment effect (7). There was no evidence of any oncogenic effect from any

TABLE 3. Summary of CD-1 mouse teratology study results (litter averages).

Diet	CLD	FC		TP		GAM		ELE		Positive control <sup>a</sup>
		35%	70%	35%	70%	35%	70%	35%	70%	
Litters examined	23	27	26	27	24	29	29	24	28	26
Weight, day 18 gestation (g)	47.0	46.4	51.2	48.0	48.3	47.1	46.7	50.3	48.4	46.5
Gestation weight gain (g)	20.6	20.3	23.9	21.5	22.5	21.3	21.2	23.4	22.1	20.3
Uterine examination, day 18										
Total fetuses	11.0	10.4	11.0	10.4	10.8	10.7	10.7	11.6	10.4	9.85
Live fetuses	10.7	9.89	10.8	10.2	10.5	10.5	10.2	11.3	10.2	9.31
Dead fetuses	0.304	0.481	0.231	0.148	0.333	0.207	0.414	0.292	0.250	0.538
Live fetus avg. wt. (g)	1.007	1.050	1.119	1.060	1.011	1.045	1.027	1.018	1.022	0.940
Corpora lutea	10.09	9.52	9.96	10.04	9.79	9.59	8.97	9.71	9.96	10.12
Implantation sites	11.5	10.6	11.3	10.8	11.3	11.0	10.9	11.7	11.4	11.1
Resorptions	0.782	0.593	0.462	0.629	0.667	0.518	0.586	0.792	1.143	1.42
Endometrial hyperplasia	2	0	2	0	0	0	0	1	2	1
External abnormalities (number of litters affected)										
Litters examined	23	27	26	27	24	29	29	24	28	26
Subcutaneous hemorrhage	4	0	1	3	1	1	1	1	0	1

<sup>a</sup>Positive control: 80 mg retinoic acid/kg body weight.

TABLE 4. Summary of rat teratology study results (litter averages).

Diet	CLD	FC		TP		GAM		ELE		Positive control <sup>a</sup>
		35%	70%	35%	70%	35%	70%	35%	70%	
Litters examined	29	25	31	30	28	29	31	27	28	30
Weight, day 20 gestation (g)	354	357	358	363	361	360	360	355	353	348
Gestation weight gain (g)	133	139	138	143	141	140	140	134	136	129
Uterine examination, day 20										
Total fetuses	12.7	12.5	11.6	13.1	12.3	13.1	13.2	10.9	12.7	12.1
Live fetuses	12.7	12.5	11.6	13.1	12.3	13.1	13.2	10.9	12.7	12.1
Dead fetuses	0.03	0	0	0	0	0	0	0	0.04	0.0
Live fetus avg. wt. (g)	2.72	2.81	2.88	2.77	2.85	2.75	2.71	2.86	2.82	2.43
Corpora lutea	14.5	14.1	14.5	15.0	14.2	15.0	14.6	14.1	14.7	14.6
Implantation sites	13.4	13.2	13.5	13.7	12.9	13.6	13.9	12.1	13.4	13.4
Resorptions	0.72	0.72	1.81	0.63	0.54	0.48	0.74	1.30	0.71	1.33

<sup>a</sup>Positive control: retinoic acid 7.5 mg/kg body weight.

TABLE 5. Summary of hamster teratology study results (litter averages).

Diet	CLD	FC		TP		GAM		ELE		Positive control <sup>a</sup>
		35%	70%	35%	70%	35%	70%	35%	70%	
Litters examined	21	25	23	27	24	26	23	28	25	24
Weight, day 15 gestation (g)	143	147	143	144	146	146	145	144	146	132
Gestation weight gain (g)	34.6	32.8	34.0	32.5	36.2	34.5	35.5	33.9	33.4	21.8
Uterine examination, day 15										
Total fetuses	10.24	9.56	10.09	9.85	10.46	10.42	10.09	10.25	10.32	3.83
Live fetuses	10.24	9.56	10.00	9.81	10.42	10.35	10.04	10.25	10.24	3.54
Dead fetuses	0.0	0.0	0.09	0.04	0.04	0.08	0.04	0.0	0.08	0.29
Life fetus avg. wt. (g)	1.68	1.71	1.65	1.66	1.67	1.65	1.60	1.69	1.62	1.50
Corpora lutea	10.7	10.7	10.5	10.6	11.5	10.5	10.8	10.7	10.9	11.1
Implantation sites	11.1	10.2	10.3	10.1	11.3	11.1	10.9	11.1	11.0	10.4
Resorptions	1.05	0.80	0.48	0.37	0.88	0.69	0.87	0.79	0.60	6.58

<sup>a</sup>Positive control: retinoic acid 30 mg/kg body weight.

TABLE 6. Summary of rabbit teratology study results.

Diet	CLD	FC		TP		GAM		ELE		Positive control <sup>a</sup>
		35%	70%	35%	70%	35%	70%	35%	70%	
Litters examined	11	13	17	13	17	15	14	12	13	15
Weight, day 29 gestation (g)	3767	3845	3841	3822	3782	3892	3834	3938	3758	3758
Gestation weight gain (g)	295	370	274	297	242	341	314	394	298	132
Uterine examination, day 29										
Total fetuses	6.45	7.54	7.47	7.08	7.06	7.20	7.57	7.92	7.85	6.13
Live fetuses	6.36	7.54	7.41	6.92	7.06	7.20	7.50	7.92	7.69	5.47
Dead fetuses	0.091	0.0	0.05	0.15	0.0	0.0	0.07	0.0	0.15	0.67
Avg. fetus wt.	40.3	36.6	35.2	33.8	34.7	37.0	37.0	37.8	35.7	34.3
Viable fetuses at 24 h (%)	85.7	89.6	78.9	72.8	76.3	95.7	91.6	91.7	88.4	52.5
Skeletal Abnormalities (number of litters affected)										
Missing 13th rib	5	5	10	5	5	6	8	6	3	7
Soft tissue abnormalities (number of litters affected)										
Hydrocephalus	0.0	2	1	0.0	1	2	0.0	4	0.0	

<sup>a</sup>Positive control: thalidomide 150 mg/kg body weight.

TABLE 7. Summary of 20th week body weights and 0-20 week weight gains of beagle dogs.

		Diet				
Gen	Sex	CLD	FC	TP	GAM	ELE
Mean body weight week 20 (kg)						
F <sub>0</sub>	M	11.35 <sup>a</sup>	12.91 <sup>d</sup>	11.69	11.62	11.09
F <sub>1a</sub>	M	10.56	14.32	12.35	11.87	12.23
F <sub>1b</sub>	M	10.37	13.94	12.89	12.09	12.14
F <sub>0</sub>	F	9.19 <sup>a,b,c</sup>	11.55	9.72 <sup>f</sup>	10.15	10.73
F <sub>1a</sub>	F	8.20	11.05	9.81	9.74	9.49
F <sub>1b</sub>	F	8.99	11.27	9.65	9.85	9.61
Mean body weight gain 0-20 weeks (kg)						
F <sub>0</sub>	M	9.72	10.65 <sup>d</sup>	9.79	9.79	9.18
F <sub>1a</sub>	M	8.95 <sup>a</sup>	12.24 <sup>b,c,d</sup>	10.45	9.72	10.19
F <sub>1b</sub>	M	9.01	11.76	10.81	9.96	10.16
F <sub>0</sub>	F	7.71 <sup>a</sup>	9.55 <sup>b,c</sup>	8.07	8.27	8.89
F <sub>1a</sub>	F	6.73 <sup>a</sup>	9.16 <sup>c,d</sup>	7.98	7.85	7.70
F <sub>2b</sub>	F	7.56 <sup>a</sup>	9.38 <sup>d</sup>	7.97	7.99	7.84

<sup>a-f</sup>Highly significantly ( $p < 0.01$ ) different (f-test) from comparison. Comparisons: a = CLD-FC, b = FC-TP, c = FC-GAM, d = FC-ELE, e = TP-GAM, f = TP-ELE.

of the diets. The group of F<sub>0</sub> females fed the gamma-irradiated diet had comparatively greater fecundity than dogs on other diets (Table 8). No evidence of reproductive toxicity was seen through four parities of F<sub>1</sub> litter

production with any of the test diets.

During the production of two litters of the CD-1 mice in each of the F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> generations fed diets containing chicken, the only evidence of impaired reproduc-

TABLE 8. Cumulative litter production in  $F_0$  female beagles.

Generation	Diet				
	CLD	FC	TP	GAM	ELE
	(Number of litters whelped)				
$F_{1a}$ parity	17	18	16	19	19
$F_{1b}$ parity	11	17	13	18	17
$F_{1c}$ parity	3	5	5	16	5
$F_{1d}$ parity	1	0	1	3	0
Totals	32	40	35	56	41

weight males fed diet GAM, compared to the other groups. Over 60% of mice of both sexes fed diet CLD survived to 24 months (Table 11). In the meat-fed groups, two-year survival ranged from 50% in males fed diet TP to 27% in females fed diet GAM. Survival in males fed diets FC, GAM, and ELE was approximately equal but significantly ( $p < 0.05$ ) poorer than males fed diet TP. Bred females in groups FC, TP, GAM, and ELE had similar patterns of mortality, but virgin females fed gamma-irradiated chicken meat (GAM) had significantly

TABLE 9. Numbers of mice bearing zero, one, or two litters.

Generation	Litters	Diets				
		CLD	FC	TP	GAM	ELE
$F_0$	0	4	4	1	1	2
	1	3	13	7	8	6
	2	33	43	32	31	32
Total		40	60	40	40	40
$F_{1b}$	0	1	1	4	1	1
	1	4	12	2	6	3
	2	23	28	17	21	23
Total		28	41	23	28	27
$F_{2b}$	0	1	2	0	2	2
	1	1	5	8	4	1
	2	20	17	8	11	19
Total		22	24	16	17	22

TABLE 10. Body weights in  $F_0$  mice at weeks 15 and 35: breeders vs. non-breeders.

Time	Sex	M					F				
	Diet	CLD	FC	TP	GAM	ELE	CLD	FC	TP	GAM	ELE
Week 15											
Breeders (g)		38.50	39.35	37.88	37.78	38.53	31.05	30.53	30.80	30.28	31.13
Non-breeders (g)		38.60	39.35	37.24	37.10	37.74	30.99	30.47	30.29	30.04	30.84
Week 35											
Breeders		38.35	42.90	41.90	43.77	42.55	35.22	38.38	39.80	39.34	39.20
Non-breeders		40.35*	45.08	43.42	42.52	43.52	32.44**	35.73**	35.50	36.02**	34.68**

Significantly different from breeders (student t-test): \* $p < 0.05$ , \*\* $p < 0.01$ .

tion noted was comparatively decreased fertility in mice fed diet TP (Table 9). When the groups fed irradiated chicken (GAM and ELE) were compared to the group fed the frozen control chicken (FC), no significant ( $p < 0.05$ ) differences were noted in frequency of stillbirths, numbers of viable offspring born, and survival to weaning in  $F_1$  through  $F_3$  generations.

Mice fed diet CLD had lower mean body weights throughout life than those fed the meat diets (Table 10). Many mice became obese in groups FC, TP, GAM, and ELE. Mean body weights in female mice fed the meat diets did not differ significantly ( $p < 0.05$ ). However, male mice fed diet GAM had lower mean body weights than males fed diets FC, TP, and ELE during the second year of feeding. This was due to decreased survival among heavier weight animals in group GAM, although overall survival for male mice did not differ significantly ( $p < 0.05$ ) among groups FC, TP, GAM, and ELE. No specific pathology could be associated with heavier

( $p < 0.05$ ) poorer survival than virgin females fed frozen control chickens (FC).

Non-neoplastic disease processes probably caused the poorer survival in the meat-fed groups, compared to group CLD. Myocardial degeneration and fibrosis (cardiomyopathy) were common in meat-fed mice, especially in those which died before the end of the study, while just one mouse in group CLD was found to have this disorder before terminal sacrifice. The incidence was highest in group GAM and lowest in group TP for both sexes. Immune complex glomerulonephropathy was the most important renal lesion, increasing in incidence and severity with advancing age. In male mice, the incidence of this lesion was lowest in groups CLD and TP and approximately equal in groups FC, GAM, and ELE. Time-adjusted analysis of the incidence of immune complex glomerulonephropathy in female mice indicated a statistical association between the early incidence of this lesion and significantly decreased survival in unbred females in

TABLE 11. Percent survival of  $F_0$  mice to 24 months.

Animals	Diet				
	CLD	FC	TP	GAM	ELE
Males	68	48	58	46	48
Females	56	38	40	28	44
Males (bred)	60	54	62	42	44
Males (not-bred)	74	48	56	50	52
Females (bred)	62	36	48	40	44
Females (not-bred)	50	46	42	20	42
Males (small lowest 25%)	76	52	58	68	68
Males (medium)	72	56	72	52	38
Males (large highest 25%)	54	40	50	18	54

TABLE 12. Most frequently observed neoplasms in  $F_0$  mice (affected/observed).

Neoplasm	Sex → Diet →		M	M	M	M	M	F	F	F	F	F
			CLD	FC	TP	GAM	ELE	CLD	FC	TP	GAM	ELE
Alveologenic tumor			21/108	41/168	18/113	15/112	20/109	21/108	17/166	15/113	15/112	11/111
Hepatocellular carcinoma			7/108	16/168	5/113	3/112	4/109	0/108	2/166	0/113	0/112	0/111
Hepatocellular adenoma			0/106	1/164	0/113	0/112	1/108	0/107	0/163	0/112	0/110	0/109
Lymphosarcoma			3/108	8/168	9/113	7/112	3/109	7/108	28/166	8/113	13/112	7/111
Hemangiosarcoma			1/108	5/168	3/113	3/112	4/109	2/108	3/166	2/113	4/112	1/111
Mammary adeno carcinoma			0/0	0/3	0/2	0/2	0/0	11/74	17/127	6/85	3/75	2/76
Leiomyosarcoma			0/108	0/168	0/113	0/112	0/109	2/108	5/166	6/113	5/112	4/111
Pituitary adenoma			0/86	0/112	0/81	0/65	0/77	8/86	7/121	5/83	1/81	3/86
Reticulum cell sarcoma			0/108	1/168	0/113	2/112	1/109	5/108	5/166	3/113	2/112	2/111
Kidney adenoma			1/107	2/167	2/112	0/112	0/109	0/108	0/164	0/112	0/111	0/109

group GAM, compared to the control group FC. It was not possible to make a causal connection. Atrial thrombosis was strongly associated with the concurrent incidence of glomerulonephropathy, although these two disease processes are not known to be related. The incidence of the combination was approximately equal among male groups, but it was significantly ( $p < 0.05$ ) higher in group GAM females compared to group ELE. It was not possible to determine if obesity was a factor in chronic heart and kidney disease. Overall, the pattern of the degenerative heart and kidney diseases reflected the differential survival among groups of male mice. The situation in female mice was much less obvious. Similar heart and kidney lesions were seen in the groups of females fed chicken diets, but there was no disease or group of diseases, either neoplastic or non-neoplastic, that could explain adequately the significantly reduced lifespan of un-bred females in group GAM.

The overall incidence of neoplasms was highest in both sexes of group FC mice, the frozen chicken control group (8). In female mice, group ELE had the lowest incidence of tumors, significantly ( $p < 0.05$ ) lower than group FC. Among males, the lowest incidence was in group GAM, although there were no significant ( $p < 0.05$ ) differences among the groups fed the chicken diets. Statistical analysis of the incidence of individual tumor types revealed no noteworthy treatment differences in either sex for the more commonly observed neoplasms, such as al-

veologenic tumor, lymphosarcoma, hepatocellular adenoma and carcinoma, mammary adenoma and carcinoma, leiomyoma and leiomyosarcoma, hemangioma and hemangiosarcoma, reticulum cell sarcoma, and renal tubular neoplasm.

On the whole, the studies were consistent in producing negative results in all the variety of tests performed. There were, however, some results that were difficult to explain on the basis of the available data. These were (a) the unexplained reduction in the hatchability of the eggs of *Drosophila* reared on gamma-irradiated chicken, (b) the poor survival of the virgin female mice fed irradiated chicken, (c) the body weight decrease in the dog study, and (d) the myocardial and glomerulonephropathy

in mice that were fed irradiated chicken. The significance of the *Drosophila* test results is unexplainable without further tests. It should be pointed out, however, that mammalian data from other reproductive tests did not demonstrate any consistent patterns or trends indicative of a positive reproductive effect. These latter tests have more relevance to man than *Drosophila*. The decreased survival of the female mice in the group fed gamma irradiated chicken occurred only in one sex group, and the result was only marginally significant. These results cannot be considered as treatment related. The weight loss observed in the dogs is not of toxicological significance because of the nature of the protocol that was followed for the study. Each dog was limited to 500 g of diet per day; however, some dogs consistently consumed the entire daily ration of irradiated meats whereas the control dogs did not consume all the chow control diets. The difference in body weights between the different diet groups is attributable to excessive caloric intake by the dogs that were fed chicken meat. To maintain an "ideal weight" the diets were manipulated so that selective "overweight" dogs had their food restricted and the few dogs that were underweight were allowed to feed until their body weight increased to an acceptable level. Because of these diet manipulations the changes in body weight cannot be considered as treatment related. The glomerulonephropathy, mineralization, and cardiomyopathies seen in mice fed both irradiated and non-

irradiated chicken meat control diets may be attributed to the high protein content of the chicken meat. It is difficult to speculate on the cardiomyopathy findings, but it should be pointed out that most, if not all, the mice with those pathology findings were obese. Further, a long term chicken diet is unusual for mice so any explanation would, indeed, without further studies, be extremely speculative at best.

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